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# TIPS AND TRAPS IN THE $^{14}C$ Bio-AMS PREPARATION LABORATORY (WSam 7)

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**Abstract**

Maintaining a contamination free sample preparation lab for biological $^{14}C$ AMS requires the same or more diligence as a radiocarbon dating prep lab. Isotope ratios of materials routinely range over 4-8 orders of magnitude in a single experiment, dosing solutions contain thousands of DPM and gels used to separate proteins possess $^{14}C$ ratios of 1pMC. Radiocarbon contamination is a legacy of earlier tracer work in most biological laboratories, even if they were never hot labs. Removable surface contamination can be found and monitored using swipes. Contamination can be found on any surface routinely touched: door knobs, light switches, drawer handles, water faucets. In general, all surfaces routinely touched need to be covered with paper, foil, or plastic that can be changed frequently. Shared air supplies can also present problems by distributing hot aerosols throughout a building. Aerosols can be monitored for $^{14}C$ content using graphitized coal or fullerene soot mixed with metal powder as an absorber. The monitors can be set out in work spaces for 1-2 weeks and measured by AMS with regular samples. Frequent air changes help minimize aerosol contamination in many cases. Cross contamination of samples can be minimized by using disposable plastic or glassware in the prep lab, isolating samples from the air when possible and using positive displacement pipetters.

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## 1. Introduction

The laboratory used in preparation of biological samples for $^{14}C$ AMS must be organized to maintain sample integrity with as much, or more, diligence as a radiocarbon dating laboratory. Isotope ratios in the bio-AMS lab routinely range over 4-8 orders of magnitude, from dosing solutions containing thousands of DPM per sample (≥ 1 nCi/mg) to polyacrylamide gels for protein separations with $^{14}C$ contents of 1pMC (61 aCi/mg). The chemist must stay aware of these disparities and work accordingly. However, isotopes may be present in the laboratory that are unknown to the present inhabitant and which constitute the greatest threat to reliable experiments with AMS sensitivity. These contaminants arise from three primary sources: the laboratory and its furnishings, the air, and the tools or vessels used in experiments. These are not macroscopic "contaminants" and present no danger to the experimenter or to scintillation-based research. $^{14}C$ is a frequent legacy in most biological laboratories, even if there is no known history of isotope use. Contamination is often found on routinely handled surfaces: door knobs, light switches, drawer handles, telephone receivers, water faucets, instrument lids, etc. This surface contamination is easily demonstrated and monitored using AMS, and the first task between AMS and biochemical collaborators is to establish the magnitude of this problem . Air circulation in a laboratory building is also scrutinized to detect the distribution of $^{14}C$ aerosols and vapors. Bioanalytical procedures are reconfigured to avoid sample cross-talk, even if familiar procedures and equipment must be abandoned. Container contamination is minimized by using disposable plastic, paper, or glass labware. We look at the contributions and the ameliorations of these effects.

In general, all surfaces routinely touched need to be covered with paper, foil, or plastic that can be changed frequently. Aerosols can be monitored for $^{14}C$ content using graphitized coal or fullerene soot mixed with metal powder as an absorber. The monitors can be set out in work spaces for 1-2 weeks and measured by AMS with regular samples. Frequent air changes help minimize aerosol contamination in many cases. Isolating

samples from the air when possible and using positive displacement pipetters also reduces cross talk between samples.

## 2. The Workplace

Finding a suitable AMS preparation laboratory in the biosciences requires investigative work. The biosciences typically use significant amounts of radioisotopes ($^{3}$H, $^{14}$C, $^{32}$P, $^{35}$S, $^{125,131}$I) and contamination is generally more widespread at these facilities than in geochemistry labs. The first step is to discover as much history of the available space as possible. It is obviously best to avoid any lab previously used for isotope tracing with high levels of isotopes. A new building with a dedicated AMS suite is best, but moderate isotope contamination is acceptable when the experimenters know where it is and how to avoid its incorporation into their samples. Taking "swipes" of surfaces is a primary tool in this process.

Small glass fiber filters (e.g. Whatman GF/A, 21-24 mm) are wetted with alcohol and swiped or lightly rubbed over a few $cm^2$ of the object under test. Removable contamination and surface dirt is picked up on the swipe, which is placed in a sealed tube with low level carbon carrier and converted to graphite [1]. We add tributyrin carrier containing 1.2 mg carbon with an isotope ratio of 0.6 fCi/mg to provide enough carbon for graphite production and measurement. Surfaces touched by human hands become contaminated over the years as workers carry previously undetectable contamination between laboratories on their gloves or hands. Work bench areas receive such human carryover but are also recipients from contaminated equipment, trays, jars, etc. that are placed on them. Unremembered or unreported spills, however small and however meticulously cleaned, can leave picoCuries of $^{14}$C on a bench where AMS samples will contain only attoCuries. The bottoms of sinks often have very "high" levels of $^{14}$C (hundreds of picoCuries) that need not result from egregious ignorance of disposal regulations. The limit for non-radioactive disposal in the United States is 50 nCi/g for

certain materials (10CFR20.2005), a level that is 1000 times greater than a "hot" AMS sample. We have even found clean laboratories to which the exterior door knob is contaminated. Dusty areas also need to be swiped. Dust at vents or on the tops of shelves absorbs aerosol contaminants and integrates the aerosol history of a proposed space since the last thorough cleaning. Swipes results are semi-quantitative since we don't know the amount of carbon we picked up on the swipe or its $^{14}C$ content. If the 1.2 mg of carrier carbon dominates the swiped carbon, an estimate of the $^{14}C$ from the swiped area is obtained from the ratio expressed in pMC by multiplying with the carrier mass and a suitable unit conversion while dividing by 100. Various levels of concern and the amount of $^{14}C$ swiped from the usually few $cm^2$ area are shown in Table 1.

Note that less than 10 femtoCurie (fCi) of $^{14}C$ will double the natural $^{14}C$ in a small tissue sample and would be 1000 times background for a piece of gel sample, while an undetectable 1 dpm surface contamination represents 450 fCi. Our institution has an active instrument loan and furniture or computer recycle program. We have learned to swipe all such equipment before brining it into the AMS laboratories or offices, especially the geochemical $^{14}C$ preparation laboratories.

Airborne contamination is detected using sorbant carbon with high surface area and low isotope ratio (< 0.2 pMC). Graphitized coal [3] or fullerene soot (Alfa Aesar product #40971) mixed with fine metal powder (e.g. 325 mesh Fe) are much more absorbent than activated charcoal and are used directly as AMS samples. The "soot" is completely equivalent to filamentous graphite that is generally used for $^{14}C$-AMS [4], is inexpensive, and can be purchased rather than occupying graphite preparation time. We press the soot/metal powder into sample holders, wrap them in Al foil to prevent particles from entering the target, and leave in an air space for 1 or 2 weeks. They are then measured for 7-10 repetitions of 30 seconds. The isotope ratio usually drops as the surface is sputtered away by the Cs beam [5]. A continuously high isotope ratio (>10 pMC) indicates a routine source of $^{14}C$ in the monitored area. We discovered a forgotten jar containing $^{14}C$-labeled

diesel fuel in this way. The potential laboratory is monitored long enough to determine if the $^{14}C$ source is momentary or chronic and, if chronic, is moitored until the source is found. If the soot surface has a high ratio but the ratio drops quickly, we interpret this as a "spike" exposure sometime during the monitor period that did not have time to permeate the entire carbon absorber. Sometimes the source can be found by quizzing neighboring experimenters about their recent activitioes. Typical monitors in clean areas have elevated ratios at the surface (1-5 pMC) and drop to the baseline after being sputtered for 150-200 seconds.

The final step in locating a workspace is to develop some form of access control to prevent contamination brought in by people unfamiliar with the the required levels of caution. We dedicate certain equipment (HPLC, scales, vacuum concentrators, etc.) to low carbon isotope ratios and exclude other scientists from using the instrument, borrowing the equipment, or raiding the consummable supplies. Limiting custodial and plant engineering access is also vital, since they are generally unaware of contamination issues and may service an area of high isotopes before enetring the AMS lab.

## 2. Contamination Control

No container, surface, or tool is trusted unless the user personally knows the complete history of the surface that touches the sample. The best defense against contamination is therefore the same epithelial route that protects our bodies from toxins in our gut: slough off the surface continually. Work bench surfaces are covered with a basal layer of plastic-backed absorber that is changed at least monthly. Drawer and appliance handles are wrapped in plastic or foil sheets. Touch pad control panels are operated through "Saran" wrap that is changed frequently. A new work surface of plastic-backed paper is placed over the basal bench cover before starting any work and is discarded after that use. Disposable plastic or glass labware is used, since surfaces can not be cleaned sufficiently, and a central cleaning system may even add contamination. Positive displacement pipetters

(e.g. Tri Continent MiniPet™ or Gilson Microman™) are preferable because no pipette nose cone is exposed to aerosolized samples. If conventional pipettors are used, aerosol resistant tips are required. Especial care protects the outside of the small quartz culture tube which contains the material to be combusted. The 6 x 50 mm quartz tube is held within a 10 x 70 mm culture tube. This tube is handled in the preparation procedure, but even it is placed within a 12 x 100 mm culture tube for placement in a centrifuge. Centrifuge rotors often become contaminated by broken tubes. Finally, workers' gloves need to changed very frequently. It is typical to use an entire box of powderless gloves in a few hours. There is often resistance to the amount of disposable supplies consumed during sample preparation, but the cost of a single AMS measurement often exceeds that of the lab ware associated with the generation of 50 samples.

Tasks must be segregated to protect the preparation of low activity samples. Stock solutions with high activities are kept in a separate building that is well removed form the AMS labs since such stocks are typically purchased containing hundreds of microcuries. Typical activies in this lab include preparation of dosing solutions and checking purity of labeled chemicals through a dedicated HPLC. The AMS chemistry laboratories also segregate the individual tasks: tissue packaging, chemical treatments/purifications, drying, isotope dilution, graphite conversion, loading sample holders, etc. Graphite is most sensitive to aerosol and vapor contamination and should be done in a cleaner area than the sample preparation laboratories. An office area upwind of all $^{14}C$ sources at the facility is best and can not be in the chemistry laboratory.

## 3. Monitoring Status

The ambient atmosphere of each prep area is monitored using the soot or coal described above. Laboratories with high air exchange are best for AMS preparation because spikes of aerosol or vapor quickly flow out. We prefer to expose monitors for 2-

weeks and measure them weekly, so that two monitors are always in each location. $^{14}C$ fluctuations are usually attributed to known activities, such as defrosting freezers or working with volatile labeled compounds. Figure 1 depicts a 3-month record of multiple measurements of aerosol monitors from one of our prep areas. Most targets drop to the baseline ratio after sputtering for 150-200 s. The monitors with the highly elevated ratios in Fig. 1 were in service during a $^{14}C$-benzene inhalation experiment in a neighboring building. The monitors are tools for explaining contamination in low level samples and observing shifts in the ambient $^{14}C$ levels. They are not used to correct processed samples, but to alert the chemist to periodic or evolving problems.

Controls establish baselines from which effects are measured. AMS controls reflect the isotope concentration of undosed or predose tissues or the carbon carrier [6]. The contemporary $^{14}C$ level is well known and easy to measure precisely in neat tissue. Some extracts are too small for proper graphite production without added carrier but contain significant carbon that affects the isotope ratio. Both sample and carrier masses must be known to find the sample's isotope concentration in this case [6]. We observed a 30% offset and relatively wide scatter in isotope ratios when measuring carrier-supplemented extracts (Fig. 2) which we attribute to poor measurement of the extract mass, traditionally done by UV absorbance with a spectrophotometer. Contemporary tissue is also an excellent predose monitor of blood or urine in human or animal exposure studies. Contamination of samples by low $^{14}C$ organic chemicals is also seen during processing, and contemporary tissue or extracts reveal the magnitude of this effect. The $^{14}C$ content of carrier carbon, whether tributyrin, gel, or plastic, is consistent and used often enough to establish a statistical record. This can be used to tighten the uncertainty of the carrier over the distribution of hundreds of carrier samples rather than using the 2-4 carrier blanks prepared with each set of AMS samples. The distribution of 60 tributyrin carrier blanks compiled over the course of a specific experiment lasting 16 weeks is displayed as a probability plot in Figure 3. Over this time, the carrier averaged 11.0 pMC with a median

of 10.8 pMC and a standard deviation of 1.5 pMC. Such a well established carrier serves as a pseudo-standard that monitors contaminants in the sample drying, combustion, and graphitization processes. The blank carrier samples are dried with normal samples and can detect aerosol cross-talk of hot samples. Gradual build-up of $^{14}C$ in the vacuum concentrator, the vacuum systems, or on stored glassware can be seen in this signal, prompting a lab-wide cleanup when required.

## 4.0 Conclusions

Cultivating an appreciation of AMS sensitivity and ease of cross contamination is often met with resistance, but has always been worth the effort. Segregating procedures according to isotope concentrations and curtailing movement between dosing and AMS areas can prevent many contamination problems. Frequently changed surface layers also prevent contamination. Aerosol and vapor contamination can be the most difficult to control since it can come from other laboratories or buildings and it is only monitored after the fact. Limiting exposure of samples to the atmosphere is an effective strategy. Graphite is most absorbent to aerosol contamination or vapor, whereas it can be handled near low level AMS facilities because it has no vapor pressure itself. Developing a culture of contamination awareness is vital to $^{14}C$ AMS in the Biosciences.

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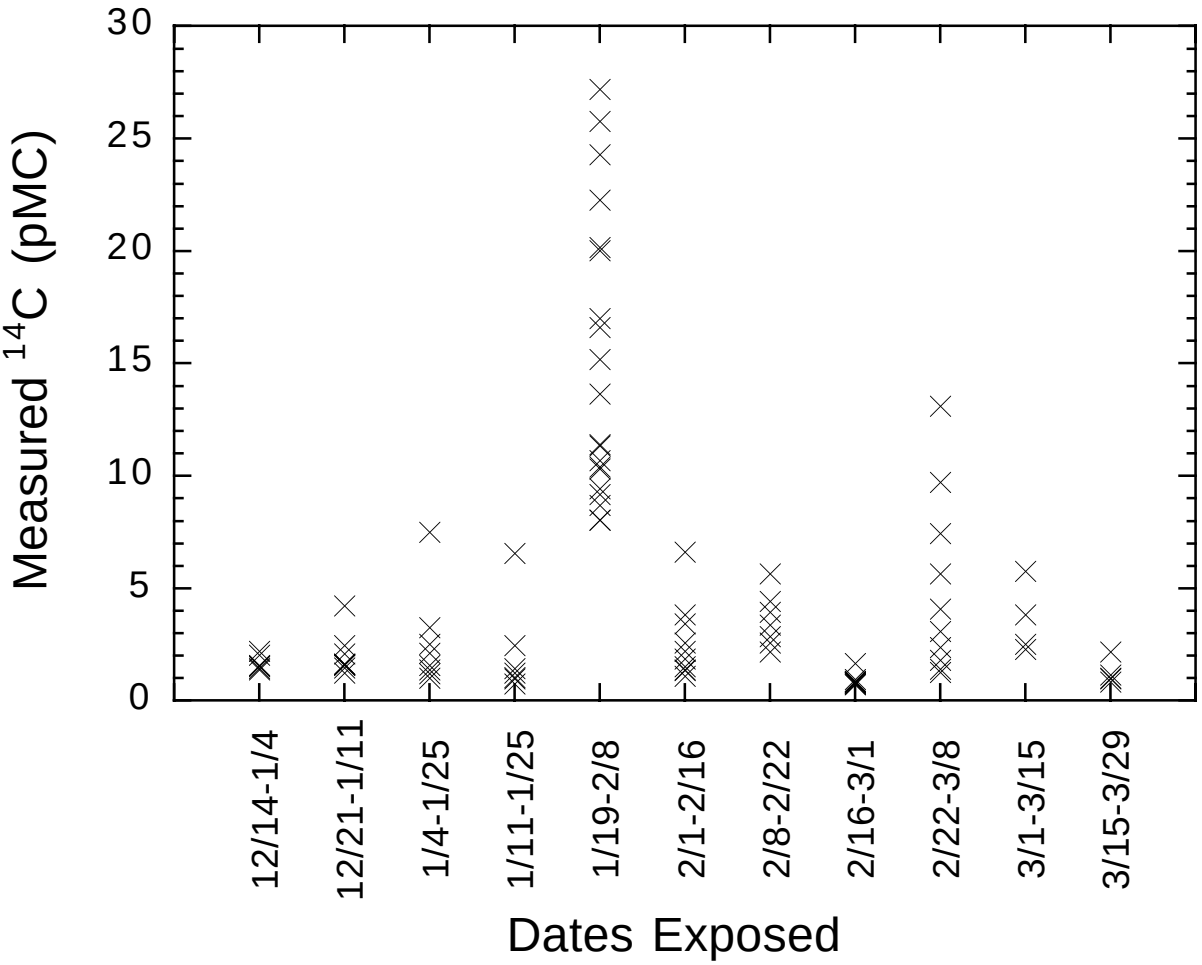
Fig. 1 Three month log of aerosol monitors in graphitization prep area. The highly elevated monitor was in service during a $^{14}C$-benzene inhalation experiment in a neighboring building.

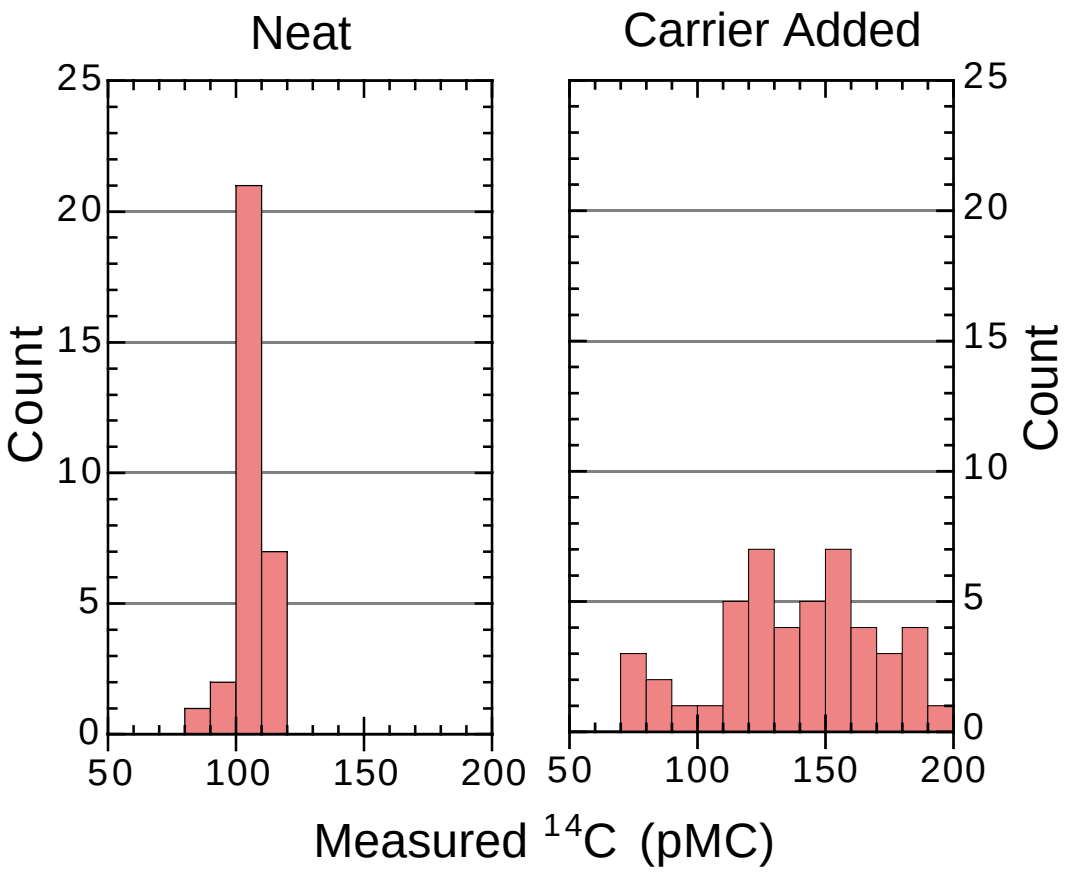
Fig. 2 Histograms of neat and carrier added contemporary control tissues. The mean and standard deviation for neat and carrier added tissue and extracts from the same animals were 107±5 pMC and 138±32 pMC, respectively. The elevation and scatter in the carrier added measurements was attributed to poor measurement of sample mass.

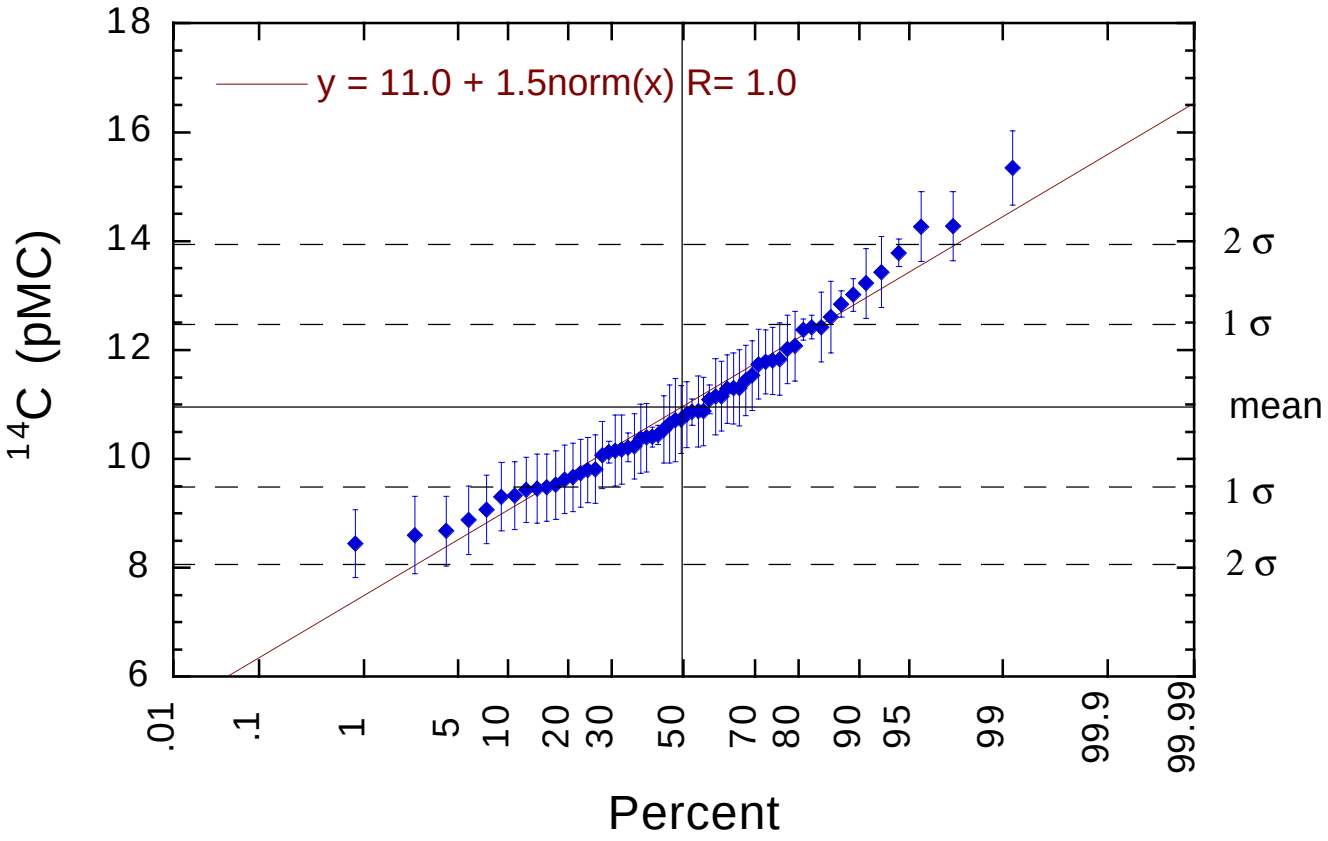
Fig. 3 Probability plot of tributyrin carrier samples (n=60) from a specific set of experiments. A distribution is Gaussian if it falls on straight line.

Table 1. The $^{14}C$ attention levels for glass filter swipes that are augmented by low $^{14}C$ carrier before measurement by AMS.

| % Modern | fCi | Conclusion | Action |
|---|---|---|---|
| 5-50 | 0.4-3.7 | Not serious | Stay alert |
| 50-100 | 3.7-7.3 | May be dirty | Clean and reswipe |
| >100 | >7.3 | Contaminated | Resurface |


Measured $^{14}C$ (pMC)
30
25
20
15
10
5
0
12/14-1/4
12/21-1/11
1/4-1/25
1/11-1/25
1/19-2/8
2/1-2/16
2/8-2/22
2/16-3/1
2/22-3/8
3/1-3/15
3/15-3/29
Dates Exposed


Neat
Carrier Added
Count
Count
25
20
15
10
5
0
50
100
150
200
Measured $^{14}C$ (pMC)


y = 11.0 + 1.5norm(x) R= 1.0
18
16
14
12
10
8
6
$^{14}$C (pMC)
2 σ
1 σ
mean
1 σ
2 σ
.01
.1
1
5
10
20
30
50
70
80
90
95
99
99.9
99.99
Percent